Please delete claims 1-5, 8 and 10

IN THE SPECIFICATION:

Please replace the paragraph on page 26 beginning on line 8 with the following rewritten paragraph:

--After addition of the cells to the media, 10 µl of N-acetyl-L-cysteine (NAC)(150 mM as final concentration) solution was added to columns 1-9 in three rows of the plates.--

IN THE ABSTRACT:

Please replace the Abstract on pages 45 and 46 with the Abstract enclosed herewith.

REMARKS

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendments. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**". No new matter has been added.

Claim 6 is amended herein to incorporate the components of the medium containing L-buthionine-[S,R]-sulfoximine (BOS). Claim 7 is amended to recite a culture medium to determine the growth rate of lymphocytes and incorporates some of the dependent claims dependent therein. Claim 12 is amended to read on a method of determining if an individual of interest has a cysteine deficiency and more specifically recites the method steps. New claims 13-15 and 16-17 are all dependent

claims and further limit the components in amended claim 6 and claim 7, respectively. As claim 6 was originally dependent on claim 1 which is cancelled herein, claims 13-15 recite some of the limitations disclosed in original dependent claims 2-5; as claim 7 has been amended, dependent claims 16-17 were added to replace original claims 8 and 10 canceled herein. Therefore, no new matter is contained in the amended and new claims.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Please amend claim 6 as follows:

6. A method of determining ~~levels of intracellular function of glutathione and analyzing biochemically cellular antioxidant function in~~ if an individual of interest has a glutathione deficiency comprising the steps of:

~~inoculating the cell culture medium of claim 1 with lymphocytes from said individual;~~

~~incubating the inoculated cell culture medium; and~~

~~comparing the response of the lymphocytes with an average response of lymphocytes from a control group of individuals.~~

1) isolating lymphocytes from said individual of interest and from more than one control individual;

2) providing a cell culture medium of a buffered, serum-free solution having a pH value from about 6.8 to about 7.6 comprising:

a) glucose;

b) a biologically utilizable form of pantothenic acid or choline;

c) at least one inorganic ion in a biologically utilizable form, wherein said ion is chloride ion, phosphate ion, calcium ion, magnesium ion, potassium ion, sodium ion or iron ion;

d) L-buthionine-[S,R]-sulfoximine (BOS), wherein said BOS is present in a concentration of about 5 μM to about 500 μM;

e) deionized water;

f) a mitogen, wherein said mitogen stimulates said lymphocytes to grow; and

g) optionally, at least one of a supplemental nutrient in a biological utilizable form wherein said supplemental nutrient is:

i) an L-amino acid;

ii) a vitamin; or

ii) at least one of pyruvate, adenine or inositol;

3) removing BSO from said cell culture medium thereby providing a BSO negative medium;

4) placing no more than half of said lymphocytes isolated from said individual of interest into BOS medium and into BOS negative medium;

5) placing no more than half of said lymphocytes isolated from said at least one control individual into BOS medium and into BOS negative medium, said media other than the media used in step 4);

6) determining growth responses of all of said lymphocytes in steps 4) and 5), said growth response measured by ^{3}H-thymidine incorporation of said lymphocytes;

7) expressing said growth response of said lymphocytes from said individual of interest as the ratio of lymphocyte growth in BOS medium to lymphocyte growth in BOS negative medium;

8) expressing said growth response of said lymphocytes from said control individuals as an average ratio of lymphocyte growth in BOS medium to lymphocyte growth in BOS negative medium; and

9) comparing said lymphocyte growth response from said individual of interest to the average growth response of said control individuals, wherein if the ratio of said lymphocyte growth response from

said individual of interest to said average control is less than about 85%, said individual of interest has a glutathione deficiency.

Please amend claim 7 as follows:

7. A cell ~~cuture~~ culture medium ~~useful for determining levels of intracellular function of cysteine and performing biochemical analysis of antioxidant function in human~~ for testing the growth rate of lymphocytes, said medium comprising:

a buffered, serum-free solution having a pH value from about 6.8 to about 7.6, said solution containing ~~the following ingredients~~:

~~a carbohydrate selected from the group consisting of~~ glucose ~~and a compound biologically capable of producing glucose in said lymphocytes,;~~

a biologically ~~usable~~ utilizable form of pantothenic acid, ~~or~~ choline ~~or a biological usable form of a substance capable of producing choline in said lymphocytes,;~~

at least one inorganic ion~~s~~ in a biologically utilizable form, wherein said ion is ~~comprising~~ chloride ion, phosphate ion, calcium ion, magnesium ion, potassium ion, sodium ion, and iron ion; ~~in a biologically utilizable form,~~

cumene hydroperoxide, wherein said cumene hydroperoxide is present in a concentration of about 5 μM to about 500 μM;

deionized water,

~~N-Acetyl-L-Cysteine~~ *N*-acetyl-L-cysteine (NAC),; ~~and~~

a mitogen ~~in an amount effective to stimulate said lymphocytes being assayed~~ wherein said mitogen stimulates said lymphocytes to grow; and

optionally, at least one of a supplemental nutrient in a biological utilizable form wherein said supplemental nutrient is:

a) an L-amino acid;

b) a vitamin; or

c) at least one of pyruvate, adenine or inositol.

~~said buffered, serum-free solution having a pH from about 6.8 to 7.6,~~

~~said cell culture medium characterized by being effective to determine nutritional deficiencies, inadequacies, and imbalances and to biochemically analyze antioxidant function of the lymphocytes.~~

Please amend claim 9 as follows:

9. The cell ~~cuture~~ culture medium of claim 7, wherein said vitamins are selected from the group consisting of biotin, folinic acid ~~or a biologically usable form of folic acid~~, nicotinamide, ~~or~~ nicotinic acid, riboflavin, thiamin, vitamin B_6, and vitamin B_{12}~~, and compounds capable of producing them in the cells; and wherein said amino acids or the compounds biologically capable of producing the amino acids comprise L-arginine, L-cysteine, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine, the amino acids being present as a group, each in an amount not exceeding inhibitory concentrations~~.

Please amend claim 11 as follows:

11. The cell culture medium of claim 7, wherein ~~the cell culture medium is~~ at least one of said pyruvate, said adenine or said inositol ~~supplemented~~ supplements said cell culture medium at

concentrations eliciting approximately a maximal response ~~with one or more stimulatory nutrients selected from the goup consisting of pyruvate, adenine, and inositol or compounds capable of producing them within said lymphocytes~~.

Please amend claim 12 as follows:

12. A method of determining if ~~levels of intracellular function of cysteine and analyzing biochemically cellular antioxidant function in~~ an individual of interest has a cysteine deficiency, comprising the steps of:

~~inoculating the cell culture medium of claim 7 with lymphocytes from said individual;~~

~~incubating the inoculated cell culture medium; and~~

~~comparing the response of the lymphocytes with an average response of lymphocytes from a control group of individuals.~~

1) isolating lymphocytes from said individual of interest and from more than one control individual;

2) providing the cell culture medium of claim 7 (NAC medium);

3) removing NAC from said cell culture medium thereby providing a NAC negative medium;

4) placing no more than half of said lymphocytes isolated from said individual of interest into NAC medium and into NAC negative medium;

5) placing no more than half of said lymphocytes isolated from said at least one control individual into NAC medium and into NAC negative medium, said media other than the media used in step 4);

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6) determining growth responses of all of said lymphocytes in steps 4) and 5), said growth response measured by ^{3}H-thymidine incorporation of said lymphocytes;

7) expressing said growth response of said lymphocytes from said individual of interest as the ratio of lymphocyte growth in NAC medium to lymphocyte growth in NAC negative medium;

8) expressing said growth response of said lymphocytes from said control individuals as an average ratio of lymphocyte growth in NAC medium to lymphocyte growth in NAC negative medium; and

9) comparing said lymphocyte growth response from said individual of interest to the average growth response of said control individuals, wherein if the ratio of said lymphocyte growth response from said individual of interest to said average control is greater than or equal to about 127%, said individual of interest has a glutathione deficiency.

Please add claim 13 as follows:

13. The method of claim 6, wherein said vitamin is selected from the group consisting of biotin, folinic acid nicotinamide, nicotinic acid, riboflavin, thiamin, vitamin B_6, and vitamin B_{12}.

Please add claim 14 as follows:

14. The method of claim 6, wherein said L-amino acid is selected from the group consisting of L-arginine, L-cysteine, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine.

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Please add claim 15 as follows:

15. The method of claim 6, wherein at least one of said pyruvate, said adenine or said inositol supplements said cell culture medium at concentrations eliciting approximately a maximal response.

Please add claim 16 as follows:

16. The cell culture medium of claim 7, wherein said *N*-acetyl-L-cysteine is present in a concentration of about 150 mM.

Please add claim 17 as follows:

17. The cell culture medium of claim 7, wherein said L-amino acid is selected from the group consisting of L-arginine, L-cysteine, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine.

Please delete claims 1-5, 8 and 10

IN THE SPECIFICATION:

Paragraph on page 26 beginning on line 8 has been amended as follows:

After addition of the cells to the media, 10 µl of ~~N-Acetyl-L-Cysteine~~ *N*-acetyl-L-cysteine (NAC)(150 mM as final concentration) solution was added to columns 1-9 ~~In~~ in three rows of the plates.

IN THE ABSTRACT:

Please replace the Abstract on pages 45 and 46 with the Abstract enclosed herewith.

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ABSTRACT OF THE DISCLOSURE

The present invention provides cell culture mediums and methods for determining levels of intracellular concentration of glutathione or cysteine in lymphocytes, indicative of nutritional status of an individual.